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14. ABSTRACT

In this first year, we measured the effect of administering Dexamathesone intravenously immediately following noise trauma and using a 10-day taper in rats.

For each animal, we measured their auditory brainstem response and modified startle reflex to measure their ability to hear a silent gap in a continuous tone. With a chronically implanted array of electrodes, we measured neural activity across the auditory midbrain, (thought to be involved in the generation tinnitus in humans). After inducing trauma in one ear with a loud, narrow band noise, we measured over the next few days and 4 weeks post-trauma, behavioral evidence of tinnitus, while measuring changes in the response of single neurons that would normally respond to sounds near the tinnitus frequency.

For 2 subgroups of the rats, we administered Dexamethasone, the drug of choice for reducing the incidence of tinnitus in humans. For the group that was administered 5mg/kg immediately post-trauma, we found no difference in the incidence of tinnitus compared to the control group (no drug). For the group that received a taper of 5, 5, 4, 4, 3, 3, 2, 2, 1, 1mg/kg on consecutive days, with first dose immediately post-trauma, the neural activity was not much changed from the control group but the behavioral evidence for tinnitus was paradoxically a lot worse than that of the control group.

15. SUBJECT TERMS

Noise trauma, Tinnitus, Corticosteroids, Dexamethasone, Animal models

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Introduction

The effect of corticosteroids on the development of tinnitus following noise trauma

ABSTRACT:

Tinnitus is the perception of sounds when no external source is present. While it is not a single disease, but a symptom of an underlying condition, a common cause of tinnitus is inner ear damage due to trauma, medication and other factors. We recently developed a rat model of tinnitus, in which we behaviorally measure evidence of tinnitus while recording from the midbrain of the rat before, just after and weeks after noise-trauma. We can then compare both behavior as well as changes in neural responses in the Inferior Colliculus before and after trauma and possible tinnitus has been established.

For humans, there is an empirical and not always consistent body of literature on the effect of various drugs provided before or just after noise trauma, and their effect on preventing or attenuating the subsequent development of hearing loss and tinnitus. One of these drugs are the corticosteroids, normally given post-trauma to mitigate the effect of trauma (Eisenman and Arts 2000). We gave Dexamethasone at various times (right away to several days) and with different regimens (pulse or taper) to rats following noise-trauma. We report on the effect of Dexamethasone on preventing tinnitus and on its effect on the coding of sounds in the midbrain following trauma.

Abbreviations: IC = inferior colliculus of the auditory midbrain, ABR = auditory brainstem response, BBN = broad band noise

METHODS:

We implant the auditory midbrain (namely, the central nucleus of inferior colliculus, IC) of 16-week old rats with a chronic multi-electrode array. In rats needing a tapered regimen of drugs, we also implant a catheter in the jugular vein to deliver drugs precisely and reliably. Once the animal has gotten used to the implant, we measure the neural activity of single neurons over a period of a week or so. This period has proven to be more variable than we predicted, since we need to find the 8-20kHz region of the IC as it is the region most likely to be affected by the development of tinnitus. Sometimes the recordings start off in the very low frequencies (1kHz or even lower), sometimes the electrodes are immediately close to the 8kHz region. At this point

1) we verify the hearing of the rat is normal using auditory brainstem response (ABR) thresholds. Referees were concerned that the electrode implant itself might generate hearing loss and tinnitus by causing lesions in the IC

2) we densely sample the neural activity in the 8-20 or 24 kHz region of the IC

3) we measure the rats' ability of detect a silent gap in an otherwise continuous tone by the use of the startle reflex.

Once this is done, noise trauma is induced with a 116dB, 1/3 Oct band of noise centered on 16kHz (14.2 to 17.9 kHz). Immediately after trauma, and following a regimen indicated below, we gave Dexamethasone, a cortico-steroid often given to humans following trauma.

At this point, 2 regimes are usually recognized: one is the temporary threshold shift, the kind of hearing loss (muffled hearing) that is common following loud sound exposure. The other is the regime of permanent effects (if present), several weeks post-trauma. The permanent effect we are studying is the emergence of tinnitus once the temporary threshold shifts have disappeared. Therefore, in the week following trauma and 4 weeks post-trauma, we

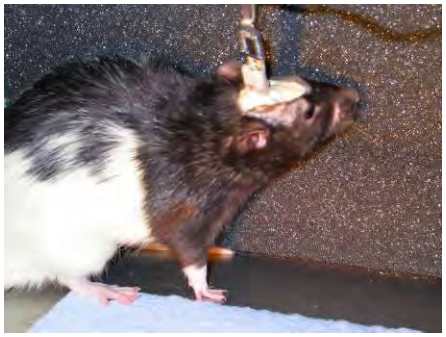
1) Verify hearing is otherwise normal as far as ABR thresholds are concerned.
2) Obtain behavioral evidence of tinnitus after 4 weeks
3) Measure changes in single neuron activity post-trauma

Note that considering the complication of keeping a jugular catheter patent, for rats receiving a single injection, we decided not use a catheter and instead are using a tail vein injection method.

PROBLEMS:

We ran into a series of unexpected problems.

1 - By far the most frustrating one, as it had nothing to do with science whatsoever but was also the most severe one, was that the implants kept on coming off. I had never had that problem at the school of medicine in Baltimore, where I developed the implant technique. It developed quite unexpectedly once I moved to the U of Maryland in College Park.



We tried to remedy the problem by changing the skull screws, the dental cement, the spacer between the implant and the skull and many other parameters. But in the end, it was simply a problem with the home cage. The food dispenser was creating a small groove or slit between it and the walls of the cage. When animals were getting stressed (cage cleaning, people walking in and out of the animal room), they would hide under the food dispenser, occasionally getting the implant stuck in that groove. Once stuck, they would try to get out and would literally yank their implants off their skull.

I apologize for giving such seemingly irrelevant details, but since the implants have to stay on the rat for at least 7 weeks for an experiment to be successful, this issue delayed me considerably. As indicated below, we are catching back up to the original schedule given in the grant proposal.

2 - Repeated anesthesia are detrimental to the health of the rats, and might be affecting the validity of the results on the effect of corticosteroids in the prevention of tinnitus following noise trauma. As it is, full anesthesia has to be given for surgery (3 hrs), first ABR (5 days post surgery), trauma (7 days), ABR post-trauma (8 days) and long-term ABR (5 weeks). Recent results by Kujawa and Liberman (but also Liberman and Dodds) have indicated both that - a normal ABR is not necessarily indicative of good hearing (as the ABR only requires enough auditory nerve fibers to be synchronized) and that – humans can present with elevated ABR thresholds, or even not have an ABR and yet have normal hearing. Unfortunately I lost a few rats to the repeated anesthesia. Therefore, after repeatedly and consistently showing that the ABR of rats subjected to the noise trauma used in this study returns to pre-trauma levels after 8 days on average, I have decided to stop using the ABR measure for now. The ABR results obtained so far in well over 20 rats has been consistent enough that no new information can be gained from it.

ABR: the animal is not just deaf

The auditory brainstem response (ABR) is *correlated* (though not unequivocally indicative, see Problem 2 above) with the health of the early parts of the auditory pathway. We measured the ABR before, in the days after and long (4 weeks) after trauma. The ABR always recovered to the pre-trauma values within 8 days.

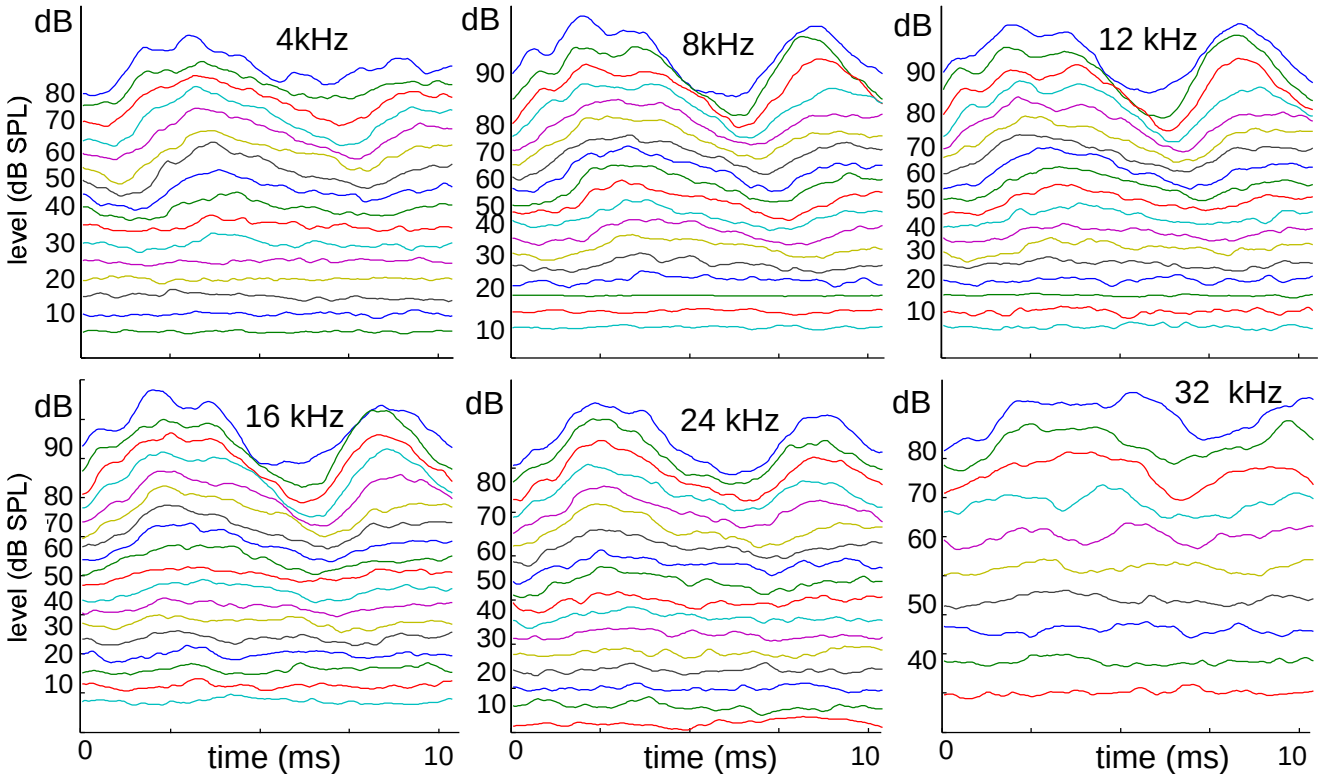


Fig.2: ABR at the frequencies indicated, 8 days post-trauma. Each frequency was presented for 10ms, 1ms ramp on and off, 800 times and averaged. An ABR threshold was measured for a sequence of tones. Threshold was defined as the minimum stimulating level where an ABR response was both identifiable and repeatable.

Behavior: Gap detection deficits as evidence of tinnitus

The ability of rats to detect a short silent gap in a continuous tone before and after noise trauma was measured, using a method similar to (Turner et al, 2006). The idea is that it is hard to hear a silent gap in an otherwise continuous pure tone if one suffers from a continuous, internally generated pure tone.

A brief burst of loud sound will elicit a “startle reflex”. The reliable presence of another stimulus just before the burst will reduce the startle reflex (this is called pre-pulse inhibition). In our case, a silent gap is present 50% of the time before the startle stimulus. We compare the startle reflex with and without a gap. This is done with a variety of frequencies for the continuous background tone.

Our noise trauma was designed previously to elicit evidence of tinnitus at just one frequency. That is, the ability to detect a silent gap in the continuous background tone should be deficient only for one frequency, which we interpret to mean that the rat has a percept of pure tone tinnitus, giving us both the most disabilitating type of tinnitus for humans and a well-defined reference o anchor point for our further neurophysiological studies.

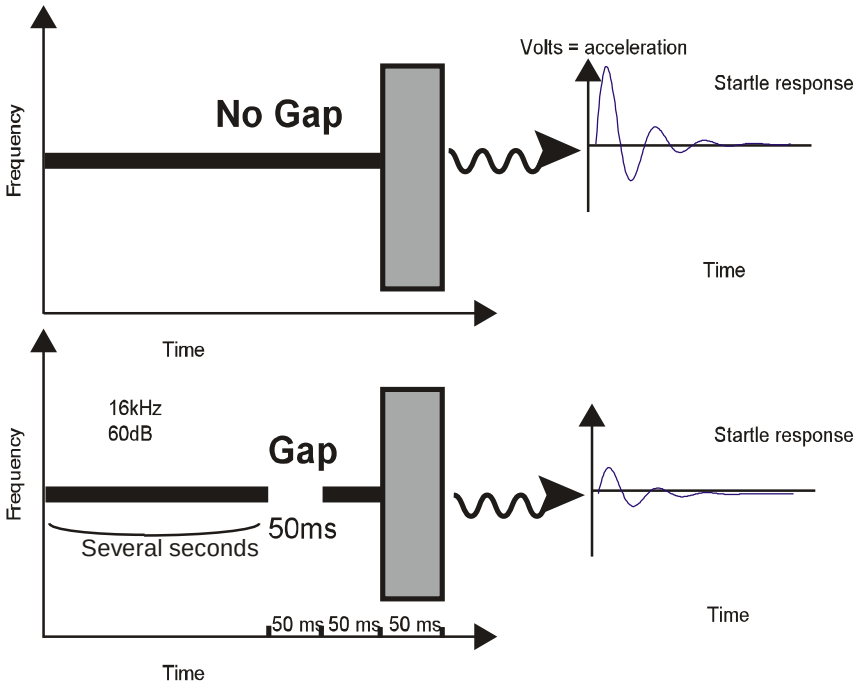


Fig.3: Startle reflex as a function of a silent gap in a tone background, measured before, just after and weeks post-trauma.

Gap detection

Practically, there's a lot of adaptation in the measurement of the startle reflex. We used 30 second long sounds so that the rats had no way to predicting when the startle stimulus would be presented. Each measurement (i.e. each frequency) was taken over 18 trials, the first 2 of which are rejected. The trials with a gap and those without a gap (nogap) are interspersed randomly. For a rat with normal hearing the ratio of gap to nogap startle reflex (whether measured by maximal amplitude or RMS of the startle response) is 0.5. For an animal unable to hear the gao, the ratio should be close to 1.

One such set of trails is shown in Fig.4. Each set is repeated 3 times (morning, afternoon and morning after).

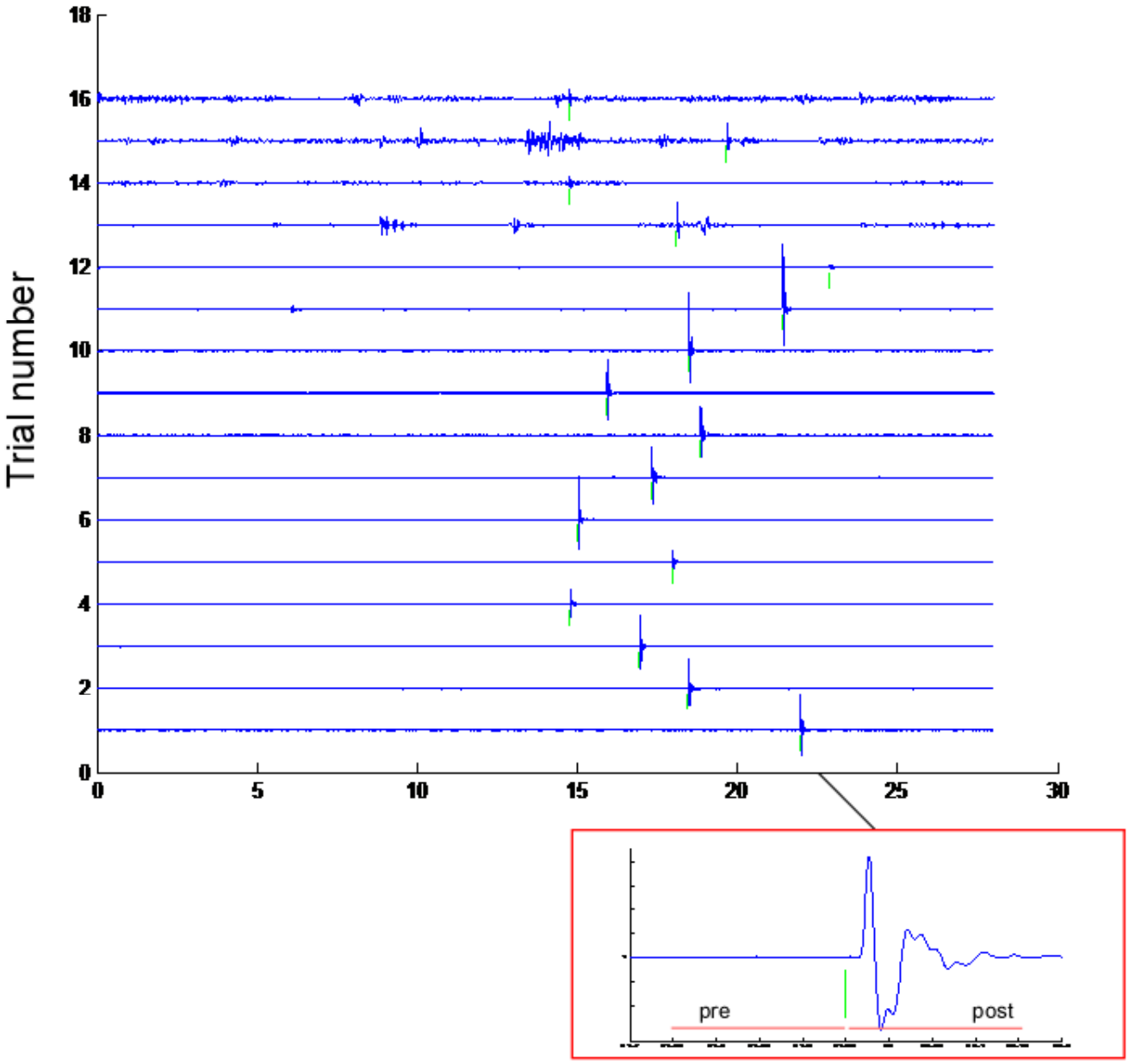


Fig.4: Our trials are 28 seconds long. The time of the startle stimulus is shown in green. Following May et al (2006), we measure the RMS of the signal prior to the startle stimulus and following the startle stimulus. We also use the maxima of the signal over the same periods, though this provides the same results. The noisy signal in the last 3 trials is due to the rat turning around in the holding tube.

Dexamethasone, 5mg/Kg immediately after trauma

We gave 5mg/Kg IV of Dexamethasone immediately after trauma or 3 hours post-trauma. As can be seen in Fig.5, **Dexamethasone given within 3 hours of trauma had no effect whatsoever on the emergence of tinnitus.**

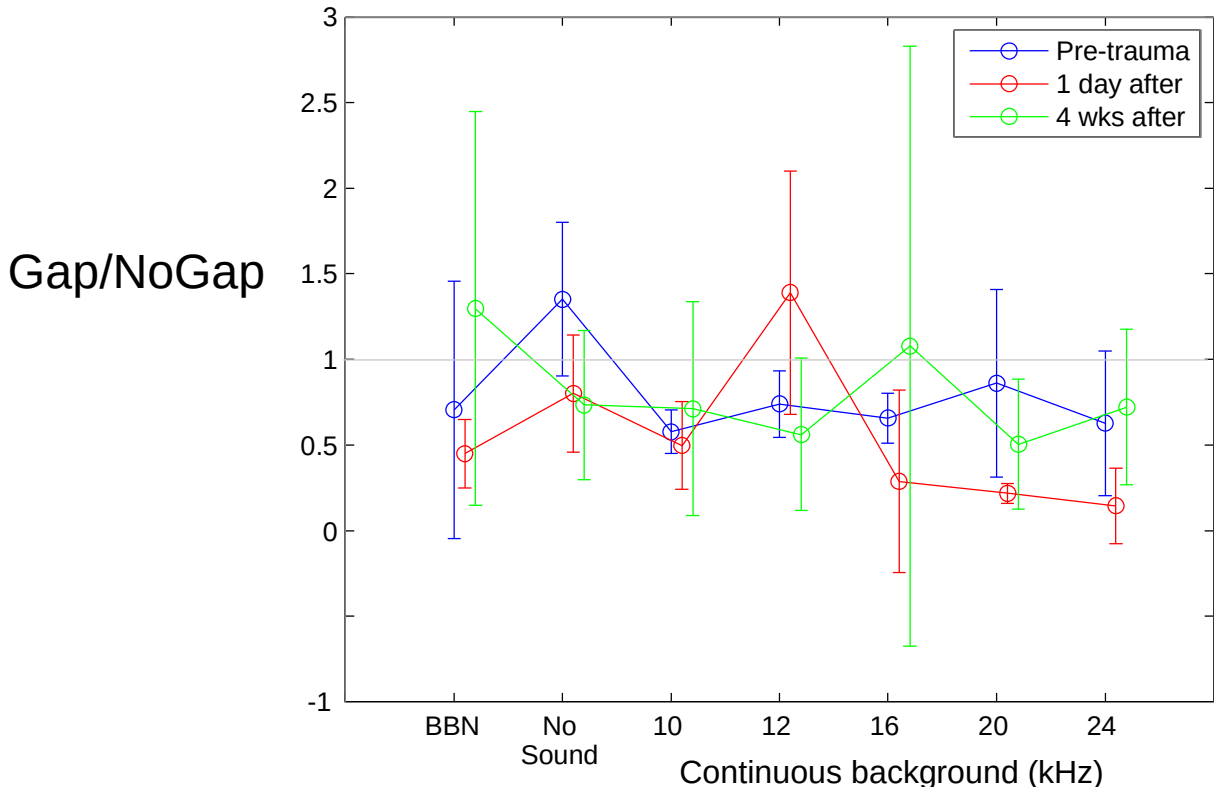


Fig.5: Dexamethasone had no effect on the emergence of tinnitus, whether given immediately or 3 hours post-trauma. Each data point is the result of 3 separate measurement done with at least 6 hours separation.

This figure requires some explanation: it shows the data for one rat, but every single rat in this condition showed the same result.

- the data shows the "gap to nogap" amplitude of the startle reflex, before (blue), in the 2 days after (red) and 4 weeks after (green) trauma.
- The BBN data point corresponds to Broad Band Noise. This measures a general ability of the rat to hear (the non-traumatized ear should be sufficient to perform the task in the presence of broad band noise. This data point would be affected only if the rat had suffered hearing damage from, for instance, a stroke resulting from the neural implant.
- The no-sound data point corresponds to a measurement in the absence of a background sound. In this case, gap and nogap are identical, i.e. this is just a sanity check. All error bars should include 1 in their range.
- The interesting results for the current grant are the ones between 10 and 24 kHz. With a trauma centered on 16 kHz is used, the is unable to hear gaps at frequencies below the frequency of trauma in the days post-trauma. This result seems to be particularly dependent on the age of the animal at the time of trauma. Our rats are 16 weeks of age and they show evidence of tinnitus only at 12kHz in the days following trauma. In a previous study in which we used 11-week old animals at the time of trauma, the evidence for tinnitus was at 10kHz.
- Once the effects of trauma have stabilized, the evidence of tinnitus is strong only at 16kHz.

Since the animals (n=5) in the group of rats that received Dexamethasone just after trauma all displayed evidence of tinnitus at 16kHz, it must be concluded that **Dexamethasone given immediately post-trauma has no effect on preventing the emergence of tinnitus. Similar results were obtained for animals receiving Dexamethasone 3 hours post-trauma.**

- As can be seen, the error bar at the 16kHz green point is very big. The physiological evidence of tinnitus (see below) points to tinnitus being present only at 16kHz, so the error bar at 16kHz is hard to interpret. However, it is know that the easiest way to induce variability in a startle reflex

experiment is to stress the subject. Therefore, it is safe to assume that the rat is stressed when hearing a 16kHz tone. This relates well to results of Melcher et al, who showed that tinnitus is often associated with hyperacusis (hypersensitivity to sound). The presence of the continuous 16kHz tone at 65dB in the background is leading the rat to be very uncomfortable, and possibly in pain. This is my interpretation of the large error bar at 16kHz.

Dexamethasone, tapered, post-trauma

We gave a Dexamethasone tapered regimen (5, 5, 4, 4, 3, 3, 2, 2, 1, 1mg/Kg on subsequent days) after trauma.

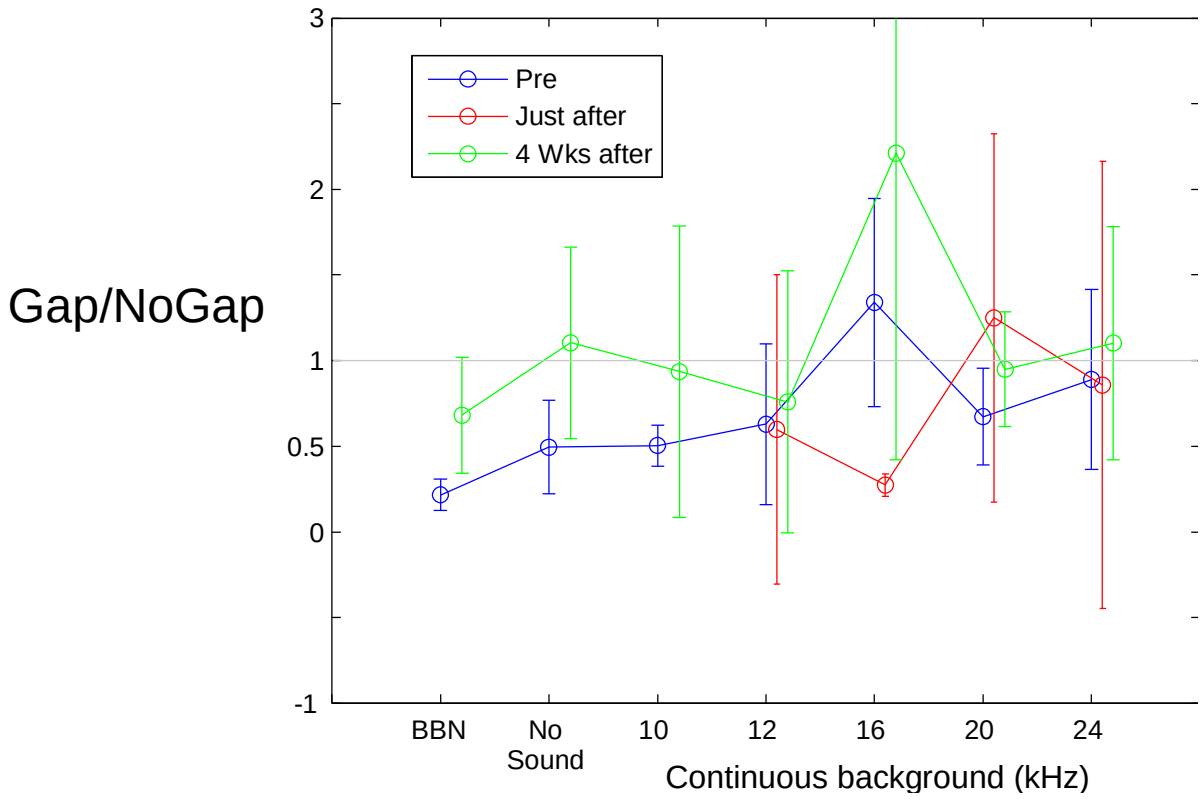


Fig.6: Dexamethasonegiven as a taper over 10 days had no effect on the emergence of tinnitus, and might even have further impaired the rat's ability to perform in the gap/no-gap test. Each data point is the result of 3 separate measurement done with at least 6 hours separation. N=1.

From the point of view of the grant, the results are even more striking. Giving a Dexamethasone taper starting just after the trauma not only did not prevent tinnitus, it seems to have affected the rats hearing at most multiple frequencies. If anything, the rat now seemed to have been *sensitized* at the frequency where the tinnitus was expected (16kHz).
Several caveats are in order, however: because of the problems mentioned above, I only have an N of 1 in this condition. Also, I used a starting dose of 5mg/Kg, which is the most effective dose in a variety of experiments (hence the choice), for instance in a study of J kaltenbach et al on facial nerve recovery after nerve section in rats. Given as a single dose (previous section), I did not have any deaths within 24 hours of administration. It could be that given repeatedly, this large amount of Dexamethasone affects the animal to the extent that it damages its already compromised organ of hearing.

At any rate, and keeping in mind that I only managed an N of 1 in this condition, the conclusion so far must be that **Dexamethasone given immediately post-trauma and then tapered over 10 days has no effect on preventing the emergence of tinnitus and might actually be detrimental to hearing.**

Electrophysiology: Controls

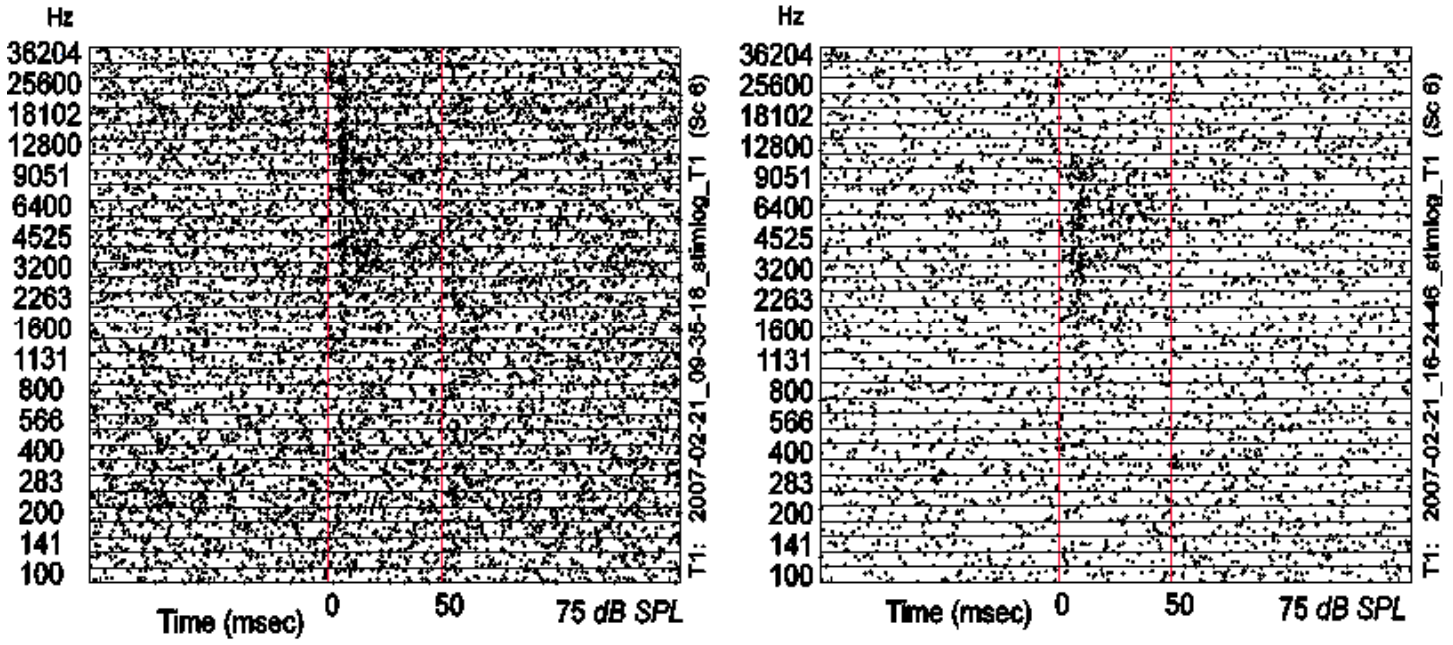


Fig.7. Tonal response in IC. Tone was on from 0 to 50ms. (Left) recording just prior to trauma, (Right) 2 hours post-trauma (no sorting). While we are probably not recording from the exact same cells, the electrodes have not been moved. Note change of neural response in the region corresponding to the trauma. The spectro-temporal receptive fields (STRFs) show the same change.

In the controls, as shown before, the effect of noise trauma and tinnitus is to 1) reduce overall level of neural activity in the period immediately following trauma. The spontaneous level of activity recovers over a period of a few days. Long term, and for neurons whose best frequency is close to the frequency of the tinnitus, there is a slight elevation of the spontaneous activity. Most notably, there is a much increased excitability of the neurons, i.e. the spontaneous activity of the neurons near the frequency of the tinnitus is much increased (by a factor of 40%) for several seconds following the extended presentation of a sound. Also, as measured by broad band sound, the bandwidth of tuning of neurons is enlarged for neurons whose best frequency was at the frequency of tinnitus, from a pre-trauma value of 1.2 octaves to a post-trauma value of 2.5 octaves.

Electrophysiology: Dexamethasone

While I will show several examples of the kind of neural activity recorded following trauma combined with Dexamethasone given just after and 3 hours post-trauma, the responses to pure tones and to broad-band sounds are statistically identical to those obtained in the controls.

Pre-trauma

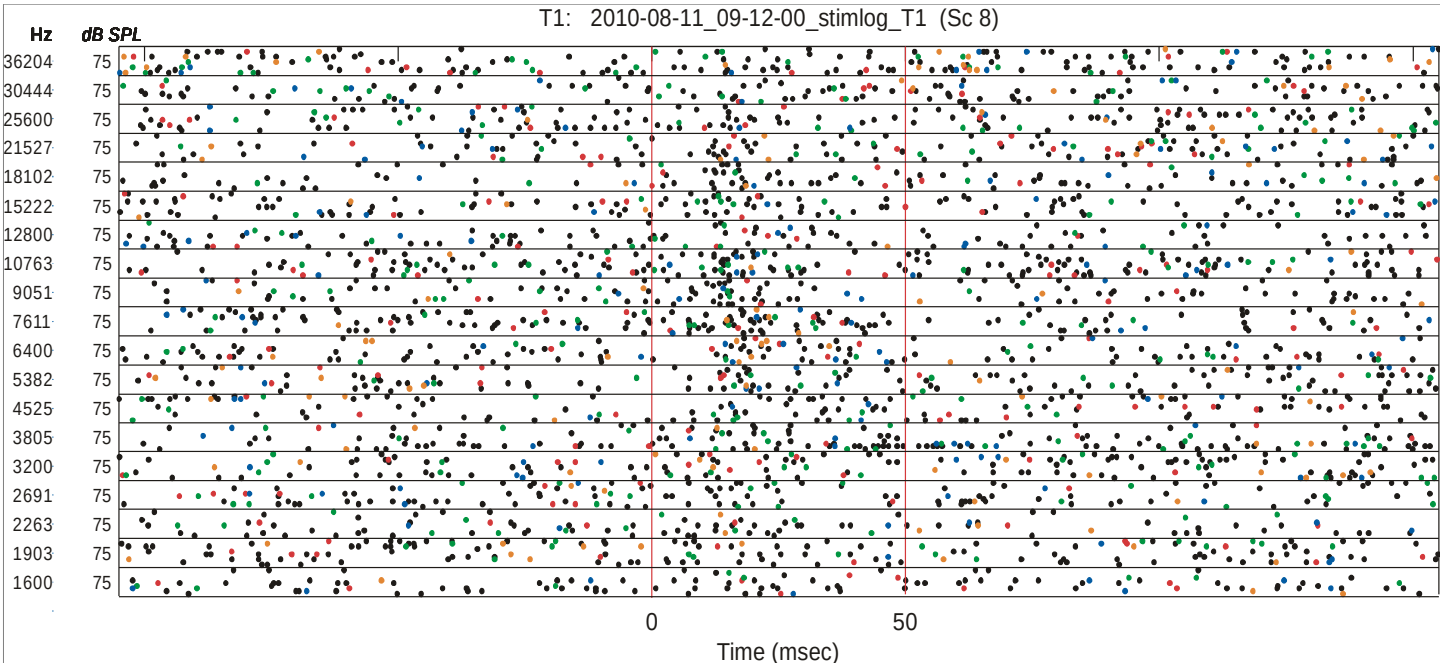


Fig.8: Same as Fig.7, for a rat about to be traumatized. Each different color corresponds to a different neuron (there were 3 in this measurement)

1 day post-trauma, Dex 5mg/Kg

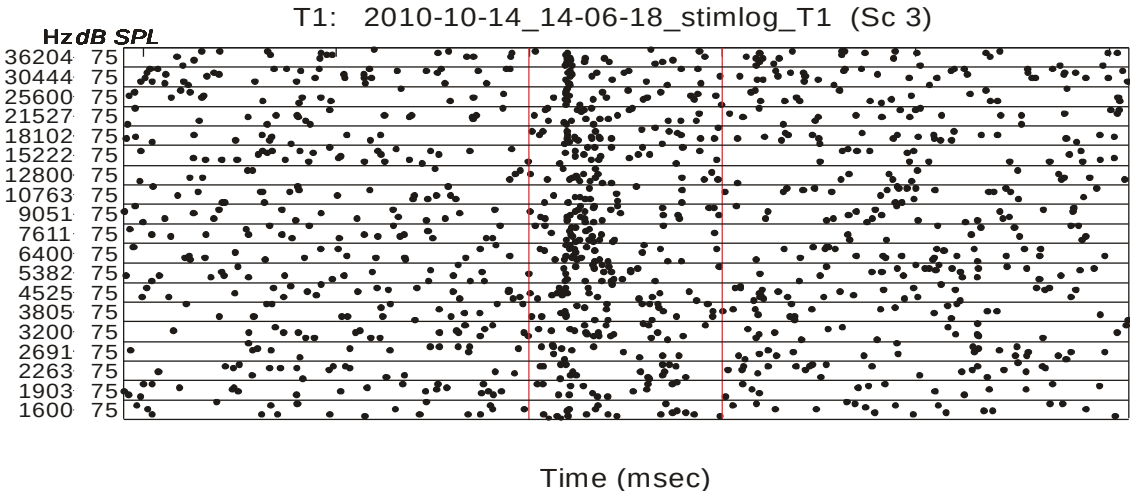


Fig.9: Overall reduced level of activity, and there are no responses from about 12 kHz to 18kHz, the frequency of the trauma.

5 weeks post-trauma, Dex 5mg/Kg

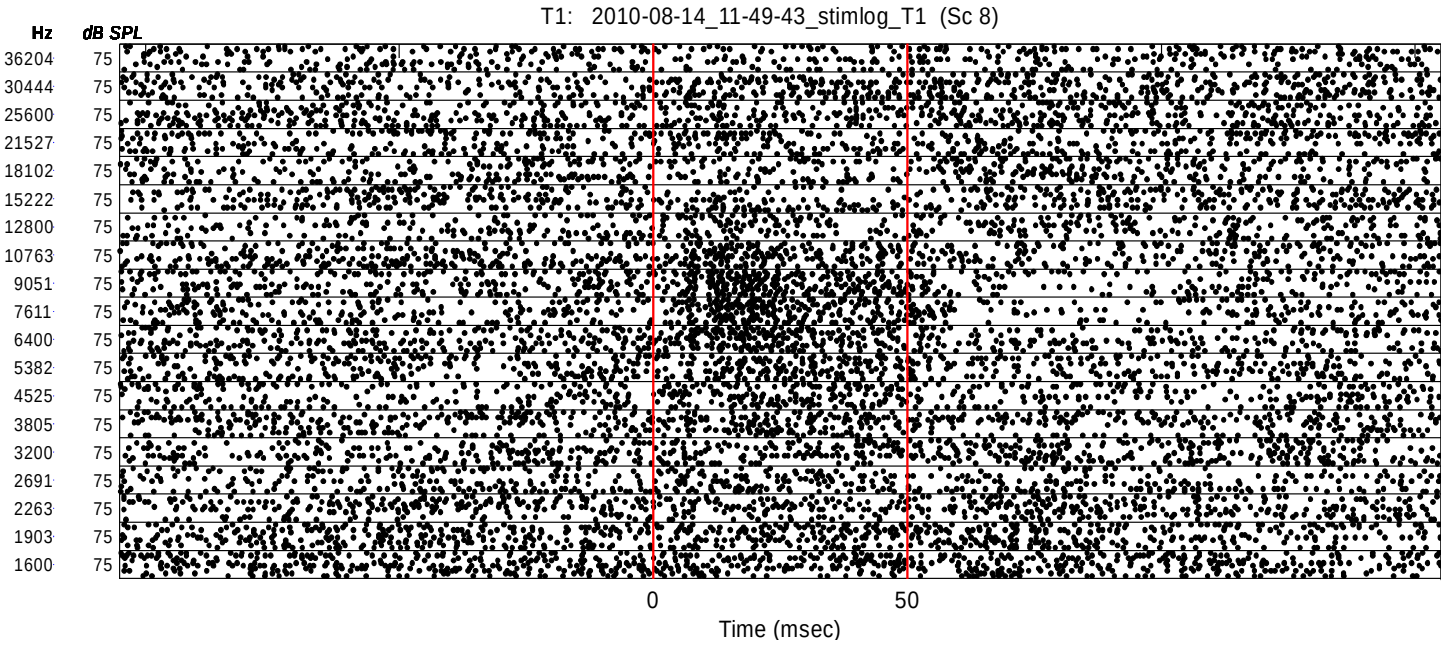


Fig.10: The spontaneous level of activity is back to the normal values, and there are no neural response at the frequency of tinnitus, 16kHz. The Dexamethasone has no measurable effect on the neural activity and on the change we have previously found associated with the emergence of tinnitus.

CONCLUSIONS OR REPORTABLE OUTCOMES

- Dexamethasone 5mg/Kg given immediately post-trauma, 3 hours post-trauma, or as a taper post-trauma does not seem to have an effect on the emergence of tinnitus following noise-trauma.

- Dexamethasone given as a taper following trauma has no preventative effect on the induction of tinnitus, and might even cause further damage, as measured with the gap/no-gap method.

However, results published or presented at conferences in the last 6 months show that 1) hair cell apoptosis does not seem to occur for at least 12 hours post-trauma (Staecker et al), and 2) Dexamethasone in mice is most effective in preventing hearing loss if given 24 hours post-trauma (Peppi eta al, Hungerford et al). Since tinnitus is strongly associated with some level of hearing loss, we will pursue our Dexamethasone experiment by giving a 5mg/Kg dose *24 hours* post-trauma, which in light of recent results I am quite confident will actually have a positive effect in preventing the emergence of tinnitus. Also, we will pursue the taper experiment, but with a much lower starting dose, and (modifying the grant proposal) we will give the first dose 24 hours post-trauma. I have talked to ENTs at the Cleveland Clinic to determine the most effective dose for the taper.

In the meantime, we have been pursuing the other major aim of the grant, namely the effect of antioxidants such as N-acetyl-cisteine and acetyl-L-carnitine given as a chronic treatment in protecting the ear against the effects of trauma.

References

Liberman MC, Dodds LW., Single-neuron labeling and chronic cochlear pathology. III. Stereocilia damage and alterations of threshold tuning curves. Hear Res. 1984 Oct;16(1):55-74.

Kujawa SG, Liberman MC. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci. 2009 Nov 11;29(45):14077-85.

Turner JG, Brozoski TJ, Bauer CA, Parrish JL, Myers K, Hughes LF, Caspary DM. Gap detection deficits in rats with tinnitus: a potential novel screening tool. Behav Neurosci. 2006 Feb;120(1):188-95.

Peppi M; Kujawa SG; Sewell WF, A Possible PLZF Link in Dexamethasone-Mediated Protection from Acoustic Trauma, poster #654, Association for Research in Otolaryngology Annual Meeting, Baltimore 2011

Hungerford M; Lei D; Lin Z.; Bao J, Development of a Combination Therapy for Noise-Induced Hearing Loss, poster #653, Association for Research in Otolaryngology Annual Meeting, Baltimore 2011

Staecker H; Gassner D; Dupanovic V; Thompson J; Imig T; Durham D, A Rat Model of Noise-Induced Tinnitus, poster #710, Association for Research in Otolaryngology Annual Meeting, Baltimore 2011